Effects of atrazine and S-metolachlor on stream periphyton taxonomic and fatty acid composition

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Abstract

Extensive pesticide use for agriculture diffusely pollutes aquatic ecosystems through leaching and runoff events and has the potential to negatively affect non-target organisms. Atrazine and S-metolachlor are two widely used herbicides often detected in high concentrations in rivers that drain nearby agricultural lands. To determine the effects of these two herbicides on river ecosystems, we conducted a 14-day laboratory experiment to expose river periphyton to a wide range of atrazine and S-metolachlor concentrations. The response of periphyton was evaluated using ecologically relevant endpoints including chlorophyll a fluorescence and fatty acids composition. Results showed that atrazine disrupted photoautotroph biomass measured by chlorophyll a fluorescence. Both herbicides caused dissimilarities in fatty acid profiles between control and high exposure concentrations, but S-metolachlor had a stronger effect than atrazine on the observe increase or reduction in saturated fatty acids (SFAs) and very long chain fatty acids (VLCFAs). Our study demonstrates that two commonly used herbicides, atrazine and S-metolachlor, can negatively affect the composition and fatty acid profiles of stream periphyton, thereby altering the nutritional quality of this resource for primary consumers.

1. Introduction

In 2020, worldwide pesticide use in agriculture was estimated at 2.7 million tons (FAO, 2022). The application of these compounds on the landscape has resulted in the detection and persistence of pesticides in aquatic ecosystems. Even at low concentrations, pesticides can interact with other compounds and represent a serious risk to aquatic and terrestrial organisms. Pesticides that target autotrophs (i.e., herbicides) represent about 48% of the pesticides used globally. Atrazine and S-metolachlor are two herbicides, commonly applied for grain, legume and cereal crop production. Resultantly, these herbicides are frequently detected in nearby aquatic ecosystems with atrazine concentrations reaching upwards of hundreds $\mu g.L^{-1}$ in agricultural regions of the USA (Hansen et al., 2019). S-metolachlor is also commonly applied for corn and soybean production, and can reach concentrations between 5 $\mu g.L^{-1}$ and 50 $\mu g.L^{-1}$ in agricultural regions of Europe (Griffini et al., 1997; Kapsi et al., 2019; Roubeix et al., 2012; Székács et al., 2015; Vryzas et al., 2011), and up to 100 $\mu g.L^{-1}$ in agricultural regions of the USA (Battaglin et al., 2003, 2000).

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] is a triazine compound marketed in the late 1950s but was subsequently banned in Europe in 2003 due to its toxicity to humans and aquatic organisms. However, atrazine is still used in several countries worldwide, including Canada and the USA, albeit under increased regulation (e.g., Quebec, see Fortier, 2018). Atrazine is a photosynthesis inhibitor herbicide that binds the D1 protein of photosystem II and blocks electron transport (Vallotton et al., 2008). Blocking electron transport, thus photosynthesis, leads to an imbalance of reactive oxygen species (ROS) causing oxidative stress, lipid peroxidation of cell membranes, and ultimately the senescence of non-crop plant species (de Albuquerque et al., 2020). When present in aquatic ecosystems, atrazine can be harmful for aquatic plants (Gao et al., 2019), micro-algae (Baxter et al., 2016), as well as non-phototrophic organisms such as bacteria (DeLorenzo et al., 1999). Moreover, atrazine is known to cause negative physiological effects to higher level organisms (e.g., amphibians, Hayes et al., 2002), and has been classified as a confirmed or probable endocrine disruptor (Pesticides Action Network (PAN), 2005).

S-metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(1S)-2-methoxy-1-methyethyl] acetamide) is an extensively used chloroacetamide herbicide available since the 1990s. S-metolachlor inhibits very long chain fatty acids (VLCFAs) biosynthesis by binding with a synthase involved in fatty acid elongation. VLCFAs are an important component for the well functioning of biological membranes. For example, Böger et al. (2003) found that S-metolachlor inhibited 68% of VLCFAs biosynthesis in the green algae *Scenedesmus acutus* compared to control. Similarly, Debenest et al. (2009) found that this compound can directly affect cellular density of periphytic diatoms. In addition, S-metolachlor is highly soluble, mobile, can bioaccumulate in non-target organisms (Zemolin et al., 2014), and it is suspected to be an endocrine disruptor for certain fish species (Ou-Yang et al., 2022; Quintaneiro et al., 2017).

Freshwater biofilms or periphyton is a heterogeneous assemblage of algae, bacteria, fungi, archaea and viruses as well as micromeiofauna trapped in a matrix of extracellular polymeric substances that develop on various submerged substrates (Wetzel, 1983). Periphyton is an integral part to the function of aquatic ecosystems and provides services in nutrient cycling. In addition, it is the basal resource of aquatic food webs providing essential compounds such as proteins, lipids and fatty acids needed for the growth and metabolism of higher trophic levels (Thompson et al., 2002). Fatty acids (FAs), in particular, are an important compound transferred along the food chain from prey to consumers (Gladyshev et al., 2011). Polyunsaturated fatty acids (PUFAs) are involved in physiological processes and maintain membrane structure (Huggins et al., 2004). While vegetal cells can synthesize PUFAs *de novo*, consumers must obtain them through dietary pathways (Brett and Müller-Navarra, 1997). In particular, certain essential FAs such as linoleic acid (LIN; C18:2n6) and α-linoleic acid (ALA; C18:3n3) are almost exclusively produced by vegetal cells; therefore, algae represent an essential source of these molecules for animal consumers (Brett and Müller-Navarra, 1997). In aquatic ecosystems, long-chain PUFAs (LCPUFAs) such as arachidonic acid (ARA; C20:4n6), eicosapentanoic acid (EPA; C20:5n3) and docosahexanoic acid (DHA; C22:6n3) are also mainly produced by microalgae (Li et al., 2014) and are transferred to consumers with high efficiency (Gladyshev et al., 2011). There is some evidence that herbicides may affect the FAs composition of microalgae by interfering with vegetal lipid metabolism (Demailly et al., 2019; Gonçalves et al., 2021). Herbicides may also induce changes in microorganism community structure of periphyton by selecting for more tolerant species that differ in FA composition (Konschak et al., 2021). For example, diatoms are known to be rich in EPA, while green algae are characterised by high content of ALA and bacteria by C18:1n9, C16:0 and C18:0. Thus, there is considerable risk that herbicides reaching aquatic ecosystems may affect the structure of periphyton assemblages and their FA profiles consequently altering the nutritional quality of this basal resource to higher consumers (Müller-Navarra et al., 2000).

In this study, we conducted a laboratory experiment to (1) determine the effects of atrazine and S-metolachlor on periphyton FA composition and to (2) relate possible modifications in FA profiles to changes in the community structure of autotrophic organisms monitored by biomass measurements. For this purpose, we exposed cultured periphyton in microcosms to either atrazine or S-metolachlor along an environmentally relevant concentration gradient.

2. Materials and methods

2.1. Experimental setup and periphyton sampling

Periphyton inocula was collected in a small stream located a few kilometers west of Quebec City (Quebec, Canada) and acclimated in the laboratory in aquaria for two months under experimental conditions (temperature = 20–22°C, natural photoperiod). Before the start of the experiment, acclimated periphyton was evenly transferred in suspension into 23 microcosms (dimensions: 30 x 15 x 20 cm) filled with 7.5 L of dechlorinated tap water enriched with nutrients (temperature = 20°C, photoperiod = 16h day/8h night, average light flux = 54 µmol photons.m^{-2}.s^{-1}, nutrients summarised in Tab.S1) and equipped with an aeration pump. Each microcosm contained six glass slides (double-sided for a total of 141 cm^2) to increase the surface area available for periphyton colonisation. After a one-month colonization period in the microcosms, periphyton was exposed to a gradient of atrazine and S-metolachlor concentrations (PESTANAL, analytical standard, Sigma Aldrich). The nominal concentrations of both herbicides tested were: 0, 5, 10, 50, 100, 500 and 1000 µg.L^{-1}. Treatments henceforth will be referred to by the first letter of the herbicide (A for Atrazine; S for S-metolachlor), followed by the nominal concentration (e.g., A5, A10, S5, S10, etc.) and the treatment that did not receive herbicide will be referred to as the control. While we employed a gradient study design with individual microcosms for each herbicide concentration, experimental replicates were incorporated for the control (n = 4), A10 (n = 3), S10 (n = 3), and S100 (n = 3) treatments. Within each microcosm, samples were collected on three occasions, before exposure (day 0), and after 7 and 14 days of exposure. Samples were scrapped from randomly collected glass slides as well as from the walls of the microcosms to make one composite sample per treatment which was preserved at -80°C for FAs analyses.

Biomass of green algae, diatoms and cyanobacteria composing the periphyton was measured with the fluorometer probe Benthotorch (bbe BenthoTorch, Moldaenke, Germany) that uses the excitation-emission responses at several wavelengths (470 nm, 525 nm and 610 nm) to determine chlorophyll a concentrations of attached autotrophic organisms. At each sampling time, six measurements were randomly taken per microcosm by placing the instrument directly onto the glass slides that were delicately and temporarily removed from the water.

Throughout the experiment, pH = 8.2 ± 0.1, conductivity = 318.3 ± 25.6 µS.cm-1 and water temperature = 18.9 ± 0.3°C (n = 66) were stable. Herbicide concentrations were determined by liquid chromatography (Finnigan Surveyor) with tandem mass spectrometry (TSQ Quantum Access; Thermo Scientific) (LC-MS/MS) (Limit of detection = 0.1 µg.L^{-1}, analytical standards: Atrazine-D5 and Metolachlor-D6). Herbicide concentrations were re-adjusted as needed over the course of the experiment. To determine any abiotic loss of atrazine and S-metolachlor in microcosms, three microcosms without periphyton were contaminated with atrazine at a nominal concentration of 500 µg.L^{-1} and three additional microcosms were contaminated with 50 µg.L^{-1} of S-metolachlor. Water was sampled after 7 days and analysed by LC-MS/MS following the same method as described above. Measured atrazine concentrations were close to nominal concentration in biotic microcosms, while S-metolachlor concentrations were below targeted values (herbicide concentrations in biotic and abiotic conditions are summarised in Tab.S2). Despite the fact that measured concentrations deviated from the targeted nominal concentrations, a concentration gradient was observed for both herbicides.

2.2. Fatty acid analysis

Fatty acid extraction and analysis were performed according to Fadhlaoui et al., (2020), where a 40 mg subsample of periphyton was homogenized in 8.4 mL of chloroform/methanol (2v/1v) solution for 1 minute using a Homogenizer 850 (Fisherbrand™). A volume of 20 µL of trycosilic acid (C23:0) was added as an internal standard and the samples were then sonicated for 5 min using a Sonifier® (Branson). A 2 mL solution of NaCl (0.73%) was then added followed by centrifugation of the sample for 15 min at 3000 tr/min at 4°C allowing for lipid separation in the lower phase. Lipids were recovered from this lower phase and evaporated using a TurboVap® (Caliper Life Sciences TurboVap II) for 15 min at 40°C before being transferred to screw-capped tubes with 3 mL of BF_3 (boron trifluoride-methanol solution 14% in methanol). The BF_3 is used to esterified fatty acids and to facilitate analysis by gas chromatography. After a one-hour incubation at 75°C, fatty acids methyl esters (FAMEs) were extracted by adding 3 mL of ultra-pure water and 3 mL of petroleum ether. This step was repeated two more times to improve FAMEs recovery. The top fraction of petroleum ether was recovered and dried using the TurboVap® for 15 min at 40°C.

Finally, FAMEs were dissolved in 240 µL of hexane and then transferred into screw-capped vials to be analyzed by gas chromatography with a flame ionization detector (Agilent Technologies; 7890D GC system) equipped with a fused silica capillary column (DB-FATWAX from Agilent Technologies: 30m [length], 0.250 mm [inner diameter], 0.25 µm [film thickness]). Injection was conducted at a constant pressure, and helium was used as the carrier gas. Temperature programming was as follows: initial temperature of 140°C increased to 170°C at a rate of 6.5°C.min^{-1}, then to 200°C at a rate of 2.75°C.min^{-1} for 14 min, and finally to 230°C at a rate of 3°C.min^{-1} for 12 min. Because the periphyton is highly heterogeneous, five subsamples from the one composite sample collected in each microcosm were analysed (pseudo-replicates) to ensure a proper representation of fatty acid profiles within each microcosm.

2.3. Statistical analysis

Statistical analyses were performed in RStudio (R version 4.2.2). Water chemistry and biomass data (µg of chlorophyll a.cm^{-2}) were expressed as mean ± standard deviation. Due to inter-microcosm variability prior to exposure, photoautotroph biomass and FA composition changes (i.e., deltas Δ) between day 0 and the two sampling times (7 and 14) were used. Delta values were then used to perform linear regressions. For all statistical analyses, results were considered significant when the p-value was less than 0.05 and marginally significant where p-value was between 0.05 and 0.1. For photoautotroph biomass, one-way ANOVAs were performed on raw data and only for replicated conditions. Pairwise t-tests with Bonferroni adjustment were used for post hoc comparisons.

Principal Component Analyses (PCA) were conducted on fatty acid data (including fatty acids with proportions > 5% in at least one sample) from pseudo-replicates, allowing the representation of intra-condition variability. The “FactoMineR” and “factoextra” packages were used to explore patterns in FA profiles as a function of exposure concentrations. A PERMutational ANalysis Of VAriance on dissimilarity matrix was performed on replicated conditions and was followed by a pairwise comparison to test for differences in FAs profiles between conditions using the ‘adonis2’ (method=’gower’) and ‘pairwise.adonis2’ functions from the ‘vegan’ package.

3. Results

3.1. Community structure of the autotrophic organisms

Effect of atrazine on chlorophyll a fluorescence

Diatoms and cyanobacteria were the two main groups of photoautotroph organisms in periphyton assemblages with green algae having a lower relative biomass (see Fig.S1 for raw data). Under the control condition, the total biomass significantly decreased between day 0 and day 14 (Df = 2, F = 9.01, p-value = 0.01). Especially, diatom biomass marginally decreased between 7 days and 14 days of exposure (Df = 2, F = 3.67, p-value = 0.05). Linear regressions showed some effect of atrazine on photoautotrophs biomass (Fig. 1). Specifically, cyanobacteria and diatoms biomass increased with atrazine concentration after 7 days (Df = 10, F = 26.44, R^2=0.73, p-value < 0.001 and Df = 10, F = 28.98, R^2=0.75, p-value < 0.001, respectively) and 14 days of exposure (Df = 10, F = 20.36, R^2=0.67, p-value = 0.001 and Df = 10, F = 25.83, R^2=0.72, p-value < 0.001, respectively). Green algae were a minor autotrophic group based on chlorophyll a fluorescence, and atrazine did not appear to affect its biomass as it remained stable between exposure concentrations and over time.

For all photoautotrophic groups, no significant differences were observed in Δbiomass between control and 10 µg.L^{-1} conditions after 7 days (Df = 5, with F = 0.68, p-value = 0.45 for cyanobacteria; F = 3.25, p-value = 0.13 for diatoms and F = 5.19, p-value = 0.07 for green algae) and 14 days of exposure (Df = 5, with F = 0, p-value = 0.99 for cyanobacteria; F = 0.15, p-value = 0.72 for diatoms and F = 0.98, p-value = 0.37 for green algae). Atrazine then appeared to have a significant effect on biomass at concentrations higher than 10 µg.L^{-1}.

Effect of S-metolachlor on chlorophyll a fluorescence

As observed for atrazine, green algae remained the minor photosynthetic group. In contrast to atrazine, S-metolachlor had no effect on photoautotroph biomass (Fig. 2).

The one-way ANOVA showed no effect of S-metolachlor at 10 µg.L^{-1} and 100 µg.L^{-1} on photoautotrophic group biomass compared to control condition after 7 days (Df = 2, with F = 1.30, p-value = 0.33 for cyanobacteria; F = 3.56, p-value = 0.09 for diatoms and F = 1.23, p-value = 0.35 for green algae) and 14 days of exposure (Df = 2, with F = 1.06, p-value = 0.40 for cyanobacteria; F = 2.02, p-value = 0.20 for diatoms and F = 0.69, p-value = 0.54 for green algae).

3.2. Effects of herbicides on periphyton fatty acid composition

Effect of atrazine on fatty acids

A total of 27 fatty acids were identified in the total lipid fraction of the periphyton. Average (+/- standard deviation) proportions of each FA are presented as supplementary information (Tab.S3). Unsaturated fatty acids (UFAs) were generally the predominant FA group in all treatments with a relative percentage of up to 62.9% comprised mostly of mono-unsaturated fatty acids (MUFAs; 23.0–41.1%) followed by poly-unsaturated fatty acids (PUFAs; 15.2–26.1%). Saturated fatty acids (SFAs) represented up to 54.3% of total lipid content in the periphyton samples.

PCA of periphyton FA relative percentages 14 days after atrazine exposure explained 66.9% of the variance in FA composition on two axes (dim1 = 42.9% and dim2 = 24%; Fig. 3). PCAs of FA composition on day 0 and day 7 are presented in the supplementary information (Fig.S2). The A5 and A100 treatments clustered together on the top left of the ordination and had higher proportions of MUFAs, in particular C16:1n7, compared to the A50, A500 and A1000 treatments that clustered in the lower portion of the PCA and were more characterized by SFAs and C18:0. A large dispersion of fatty acid data was observed, especially for the control and A10 conditions which overlapped all treatment groups.

The PERMANOVA and pairwise comparisons performed on replicated conditions (control and A10) revealed a significant difference in FA composition (Df = 1, F = 3.92; p-value = 0.02) after 14 days under atrazine exposure. PERMANOVA were also conducted at day 0 and day 7 and showed that differences were already present at day 7 (Df = 1, F = 4.88; p-value = 0.008) but not prior to exposure (Df = 1, F = 2.33, p-value = 0.08).

Linear regressions showed that atrazine concentration did not markedly affect the main FA groups (Fig. 4; linear regressions for individual FA are shown in Fig.S3). Only a regression marginally significant was observed for SFAs at 14 days (Df = 10, R^2=0.27; p-value = 0.08).

Effect of S-metolachlor on fatty acids

Mean (+/- standard deviation) proportions of each FA are presented in the supplementary information (Tab.S4). Unsaturated fatty acids (UFAs) comprised up to 62.5% of the total FA content of periphyton among treatments, while mono-unsaturated fatty acids (MUFAs) varied from 27.3–38.8% and poly-unsaturated fatty acids (PUFAs) varied from 16.9–33.4%. Saturated fatty acids (SFAs) represented up to 54.3% of total FA content in the periphyton samples.

A PCA was performed to assess the effect of S-metolachlor after 14 days of exposure (Fig. 3) (See Fig.S4 for 0 and 7 days). The first two dimensions explained 62% of the variance (dim1 = 42.1% and dim2 = 19.9%). The two highest concentrations clustered on the left side of the ordination, while S10, S100 clustered on the right side. The S5 and S50 conditions clustered on the top portion of the ordination (dimension 2) and S100 clustered on the lower half of the ordination. The control condition clustered in the middle and showed high dispersion. High S-metolachlor concentrations (S500 and S1000) were more associated with SFAs such as C18:1n9 and C18:0, while lower S-metolachlor concentrations were rather characterized by PUFAs such as ALA, EPA and C20:4n6.

At day 14, the PERMANOVA (performed only on replicated conditions; control, S10 and S100) showed a significant effect of S-metolachlor concentrations on the fatty acid composition of the periphyton. Indeed, there was a strong dissimilarity between the control and S100 (Df = 2, F = 3.79, p-value = 0.001). PERMANOVA conducted at day 0 and day 7 also revealed differences in FA profiles. Especially, the S10 condition (Df = 1, F = 4.70, p-value = 0.008) was already different from the control at day 0, while S100 had different FA composition from the control (Df = 1, F = 7.35, p-value = 0.003) after 7 days of S-metolachlor exposure. These results suggest that S-metolachlor affected the fatty acid profile of periphyton after only 7 days of exposure.

Linear regressions showed an effect of S-metolachlor contamination on the FA composition of the periphyton (Fig. 6; linear regressions for some specific FA are shown in Fig.S5). Specifically, SFAs increased along the S-metolachlor gradient (Df = 12, F = 18.70, R^2=0.60; p-value < 0.001) after 14 days of exposure. MUFAs did not vary with exposure concentrations, while PUFAs marginally decreased with increasing S-metolachlor concentrations after 7 days of exposure (Df = 12, F = 3.23, R^2=0.24; p-value = 0.08) and then significantly decreased after 14 days (Df = 12, F = 5.05, R^2=0.30; p-value = 0.04). Finally, VLCFAs decreased with increasing herbicide concentration after 7 days (Df = 12, F = 5.85, R^2=0.33; p-value = 0.03). This relationship was stronger after 14 days (Df = 12, F = 19.61, R^2=0.62; p-value < 0.001), where a delta of 10% between the highest concentration and the control was observed.

4. Discussion

Despite high variability observed in photoautotroph community structure and FA composition, results showed effects of herbicide exposure. Photoautotroph biomass tended to increase with the increase of atrazine concentration after 7 days of exposure. In contrast, S-metolachlor did not clearly affect periphyton fluorescence. As periphytic biofilms are very heterogeneous, fluorescence and fatty acid data showed large intra-condition variability. Despite marked variability, results showed that the two herbicides, in particular S-metolachlor, affected fatty acid profiles. S-metolachlor had a stronger effect than atrazine, with a greater effect after 14 days of exposure compared to 7 days. In particular, the S500 condition showed a 30% increase in SFAs and a 34% decrease in VLCFAs proportions compared to the control condition.

4.1. Effects of herbicides on photoautotrophs biomass

S-metolachlor exposure did not clearly affect periphyton biomass as measured by chlorophyll a fluorescence. Indeed, total biomass and diatom biomass decreased over time under all concentrations including the control condition. Our finding of no significant herbicide effect is in contrast with several studies that showed chloroacetamide herbicides to decrease photoautotroph growth and chlorophyll a fluorescence. For example, Thakkar et al. (2013) showed that the exposure of the marine chlorophyte *Dunaliella tertiolecta* to a high metolachlor concentration (1 mg.L^{-1}) led to a decrease in chlorophyll a and b fluorescence and inhibited cell growth. Likewise, Coquillé et al. (2015) showed a decrease in chlorophyll a content in a freshwater diatom culture (*Gomphonema gracile*) after 7 days of exposure to 100 µg.L^{-1} of S-metolachlor. The limited effect of S-metolachlor on photoautotrophic groups could be linked to the periphyton matrix that is composed by extracellular polymeric substances (EPS) which may represent up to 90% of the dry mass (Flemming and Wingender, 2010). The EPS matrix has several functional groups allowing for the sorption of nutrients and xenobiotics, but can also form a protective layer for the biofilm cells against substances such as pesticides (Melo et al., 2022). The universal decrease in periphyton biomass that we observed over time is likely due to the age of the periphyton in our experiment. The colonization time of periphyton varies between 2 and 4 weeks (Cattaneo and Amireault, 1992), and is followed by a biomass loss phase after 4 to 5 weeks (Trbojević et al., 2017). In order to have sufficient biomass for fatty acid analyses, periphyton was contaminated after more than 4 weeks of colonization and growth. As the experiment lasted an additional 14 days, the periphyton may have started a senescence phase, with potential detachment of biomass under all treatment conditions (Boulêtreau et al., 2006).

In contrast, atrazine increased diatoms and cyanobacteria biomass measured by chlorophyll a fluorescence. The increase in chlorophyll fluorescence can be linked to the mode of action of atrazine. When photosynthesis is proceeding normally, several steps contribute to the creation of an electron flow between different elements of the thylakoid membranes where the inhibition of photosystem II (PSII) by atrazine takes place. Atrazine competes with plastoquinone for the quinone binding site on the D1 protein (QB site) in PSII, interrupting the electron flow from plastoquinone QA to QB (Rea et al., 2009) leading to the re-emission of excitation energy as fluorescence (Muller et al., 2008) which is then captured by our measuring device. The increase in chlorophyll a fluorescence could also be linked to an increase of chlorophyll cell content. When exposed to atrazine, autotrophs within the periphyton may physiologically adapt to stress by increasing chlorophyll a content per cell (Pannard et al., 2009) to increase the number of photosystems. This “shade-adaptation” response may be a strategy to compensate for the inhibition of photosynthesis and has previously been documented to occur in response to other PSII inhibitor herbicides (e.g., diuron; Chesworth et al., 2004; Proia et al., 2011; Ricart et al., 2009). Given the mode of action of atrazine, the increase in chlorophyll a fluorescence could be taken as evidence of an atrazine effect on the periphyton. Future studies are needed to measure more endpoints in order to validate or refute this hypothesis.

In addition to affecting the fluorescence of photosynthetic organisms, the presence of herbicides may select for more resistant/tolerant taxa (Murdock et al., 2013), thus modifying the community structure of the periphyton (Schmitt-Jansen and Altenburger, 2005). We found that atrazine exposure increased the biomass of cyanobacteria in periphyton. Cyanobacteria may be more tolerant to atrazine and more competitive than diatoms and green algae as they have the potential to adapt to photosynthesis inhibition by the use of alternative carbon fixation pathways (Egorova and Bukhov, 2006). This is consistent with Pannard et al. (2009) who showed that chronic exposure to atrazine (0.1, 1 and 10 µg.L^{-1} for 7 weeks) led to a change in microalgal populations with the selection of opportunistic resistant species, some of which were cyanobacteria. Herbicides could also decrease competition for nutrients or increase labile carbon released after cell death further stimulating bacterial production (Downing et al., 2004).

4.2. Effects of herbicides on periphyton fatty acid composition

Herbicide exposure caused different changes in the FA composition of periphyton with atrazine having little effect on FA composition compared to S-metolachlor. In particular, periphyton SFAs increased with S-metolachlor concentrations, while PUFAs and VLCFAs decreased. The decrease in VLCFAs proportion with the increase in S-metolachlor concentration exposure is consistent with previous results from Böger (2003), who showed a 68% inhibition in VLCFAs of *Scenedesmus acutus* (green algae) after exposure to 283 µg.L^{-1} of S-mertolachlor. VLCFAs ($C \geq 20$) have a structural role in membranes (Bach et al., 2011; Vallotton et al., 2008).

S-metolachlor binds to the fatty acid elongation synthase (FAE1-synthase) and inhibits the formation of VLCFAs, which can then affect the rigidity and permeability of cell membranes, resulting in increased cell size and impaired cell division (Matthes and Böger, 2002; Thakkar et al., 2013). Even at lower concentrations of exposure (10 µg.L^{-1}), Demailly et al. (2019) experimentally showed that S-metolachlor significantly increased the saturated fatty acid C16:0 and decreased PUFAs including C18:4n3 and C20:4n6 of the diatom *Gomphonema gracile* after one week of exposure. The loss of PUFA observed here and in past studies may be due to the ability of S-metolachlor to increase ROS production (e.g., singlet oxygen 1O2) resulting in the peroxidation of unsaturated fatty acids in lipid membranes. More specifically, these ROS remove hydrogen from the unsaturated chain of PUFAs constituting the lipids, leading to the loss of membrane integrity (Maronić et al., 2018), in turn jeopardizing the functioning of the cell (Garg and Manchanda, 2009). In response to stress, algae often produce triacylglycerols (TAGs)

(Nakamura and Li-Beisson, 2016; Shanta et al., 2021). TAGs are considered as carbon and energy storage products (Morales et al., 2021) and are used to maintain bioenergetic stability in the cell. SFAs and MUFAs such as C16:0, C18:0 and C18:1 are among the main components of triacylglycerols (TAGs). The increase in SFAs (e.g., 18:0) and the decrease in some long-chain UFAs that we observed in our experiment could therefore suggest a protective response of the cells against membrane S-metolachlor damages (Kabra et al., 2014).

Herbicides can also have an indirect effect on the FA composition of periphyton by altering the taxonomic composition of periphyton communities. Indeed, different taxonomic groups in the periphyton complex have different fatty acid profiles. For example, diatoms are particularly rich in EPA (C20:5n3) (Drerup and Vis, 2016) and green algae are rich in ALA (C18:3n3) (Genter and Lehman, 2000), while the SFA C16:0 (palmitic acid) is important for the structure of phospholipid membranes in prokaryotes (Rock, 2008). Changes in the proportion of fatty acids may thus reflect herbicide-induced changes in the composition of the periphyton communities. More specifically, it is possible that the increase in SFAs with atrazine exposure may be due to an increase in bacteria resulting from reduced competition with photosynthetic organisms impacted by the contaminant as we observed increased cyanobacteria biomass by this contaminant (Figure S3). Nevertheless, this increase in cyanobacteria was hardly detectable in the FA profiles, where no significant changes in C18:2n6 and C18:3n3 were observed despite the fact that cyanobacteria are generally rich in these C18 PUFAs (Desvilettes et al., 1997). At present, it is still unclear what level of organization (i.e., from the cellular level to subtle changes at the community level) is responsible for the changes in the FA composition of periphyton highlighted by our experiment. It would then be useful to carry out further studies to use more endpoints such as specific composition and the number of cells per autotrophic group.

5. Conclusion

Periphyton has a key role in the structure and function of aquatic ecosystems. The nutritional quality of periphyton is essential for the development of primary consumers and can be used as indicator of ecosystem health (Desvilettes et al., 1997). Fatty acids are key nutritional compounds transferred through trophic interactions that are sensitive to various environmental contaminants. We investigated the effects of two commonly used agricultural herbicides, atrazine and S-metolachlor, on photoautotroph biomass and fatty acid composition of periphyton and found that the two herbicides acted differently on the periphyton photoautotroph biomass and fatty acids composition suggesting that there is no standard pattern of herbicide effects on stream periphytic communities. Fluorescence measurements provided information on changes in the relative biomass of the photoautotrophic groups (i.e., green algae, diatoms and cyanobacteria) within periphyton, however, we were limited in our quantification of heterotrophs. Considering that bacteria account for a large amount of biofilm mass (Ricart et al., 2009), are involved in nutrient cycles and can affect the fate of herbicides in water and within the biofilm, future studies should investigate the heterotrophic compartment of the biofilm, especially by DNA sequencing or the study of the metabolism of bacteria. The widespread presence of these two herbicides in rivers raises the question of their toxicity to non-target aquatic organisms and their interaction with the many other molecules present in water (i.e antagonist, additive or synergistic effects) (Glinski et al., 2018). This study supports the interest to use fatty acids as biomarkers (Gugger, 2002; Lang et al., 2011; Maltsev and Maltseva, 2021; Shen et al., 2016) in the context of pesticide effect assessment (Filimonova et al., 2016; Gonçalves et al., 2021)but also as a tool for water quality biomonitoring (George et al., 2015).

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Conflict of interests

The authors declare that they have no conflict of interest.

Competing Interests

None.

Author contributions

All authors made substantial contributions to this paper. L.M. was in charge of experimental conceptualization, laboratory experiments, sample collection, data analysis and writing. S.M. was involved in experimental conceptualization, project management, reviewing and editing. I.L. was responsible for funding acquisition, project administration and was involved in the project conception, experimental conceptualization, reviewing and editing.

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Figures

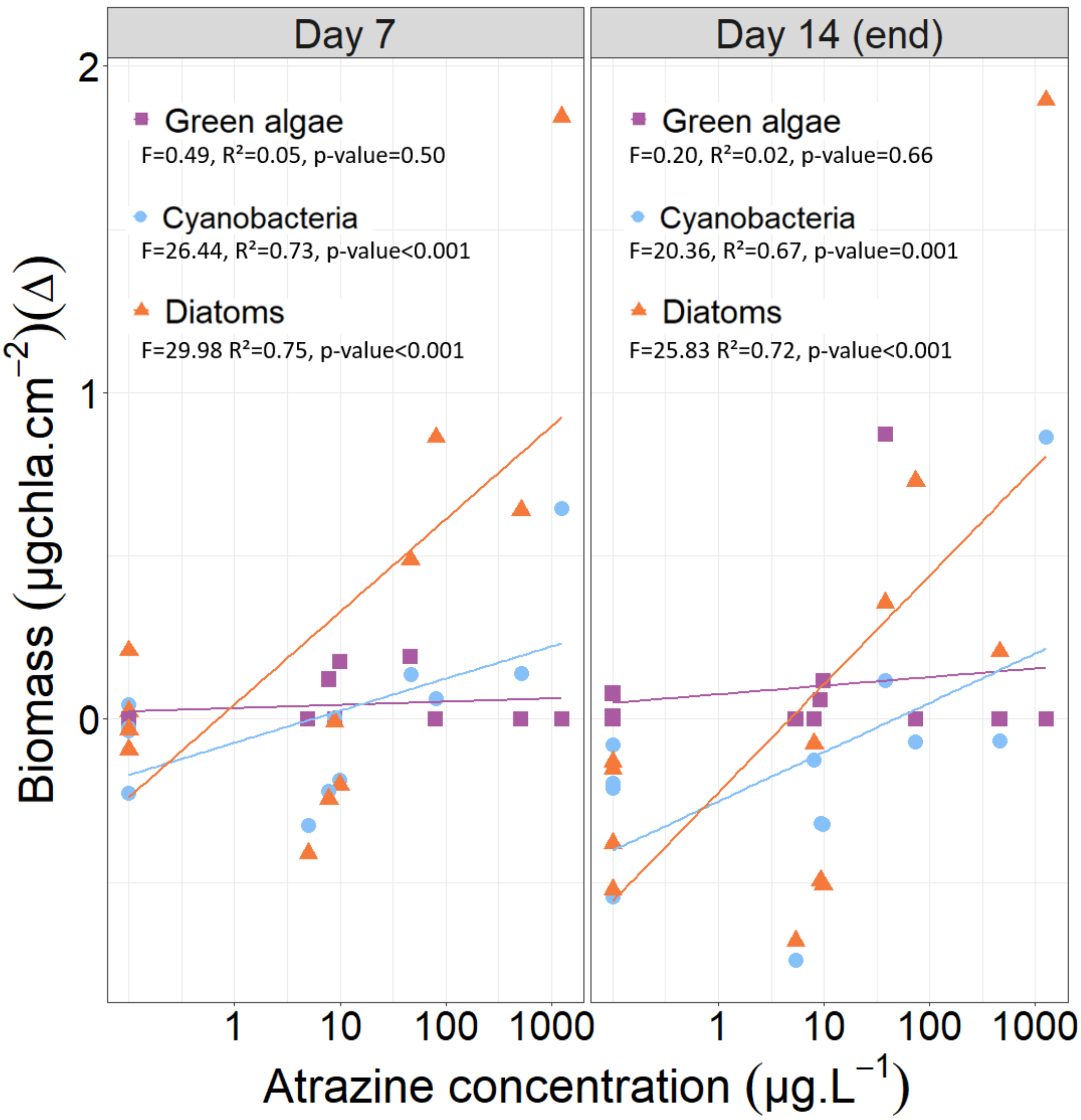

Figure 1

Biomass variation (Δ) at day 7 and day 14 compared to day 0 (initial time of the experiment), based on chlorophyll a fluorescence of photoautotrophic groups as a function of measured atrazine concentrations ($\mu g.L^{-1}$) (linear regression Df=10)

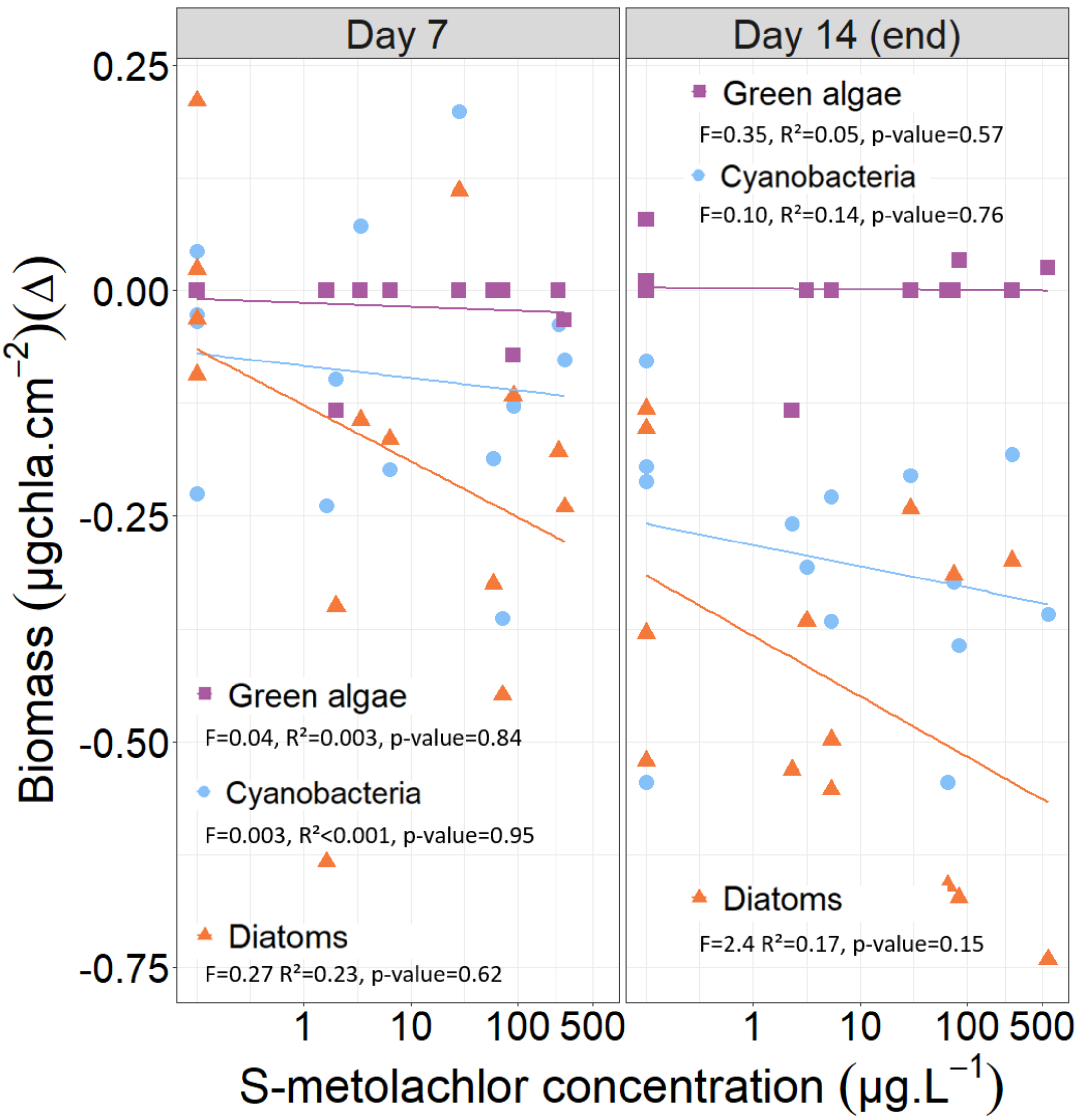

Figure 2

Biomass variation (Δ) at day 7 and day 14 compared to day 0 (initial time of the experiment), based on chlorophyll a fluorescence of photoautotrophic groups as a function of measured S-metolachlor concentrations (µg.L^{-1}) (linear regression Df=12)

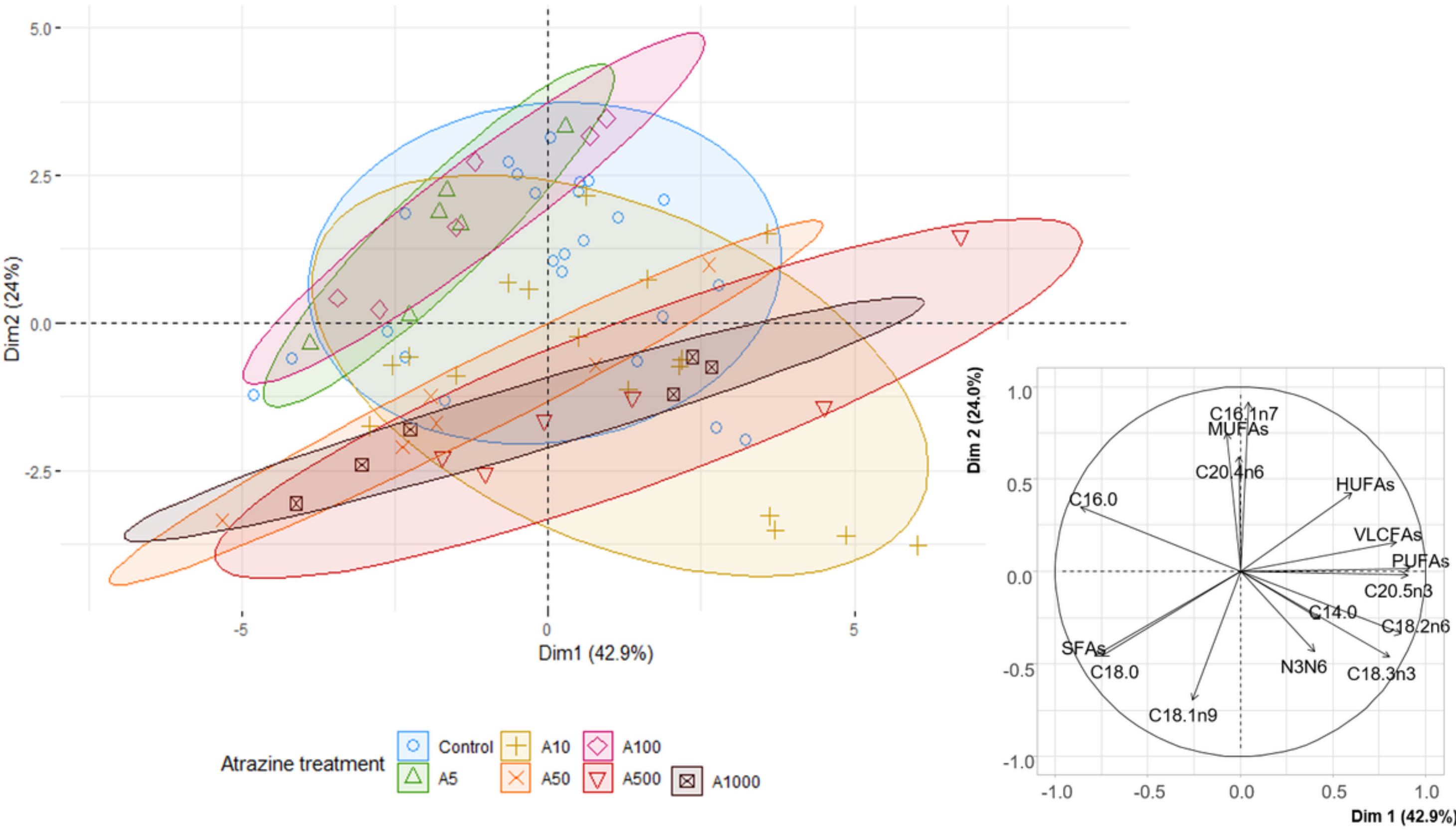

Figure 3

Principal component analysis (PCA) of fatty acid profiles at day 14 for the different atrazine conditions. The left panel is the graph of individual FA and on the right panel corresponds to the circle of correlations. Ellipses have been plotted with a confidence level of 80%. All pseudo-replicates were considered to better represent the intra-condition variability

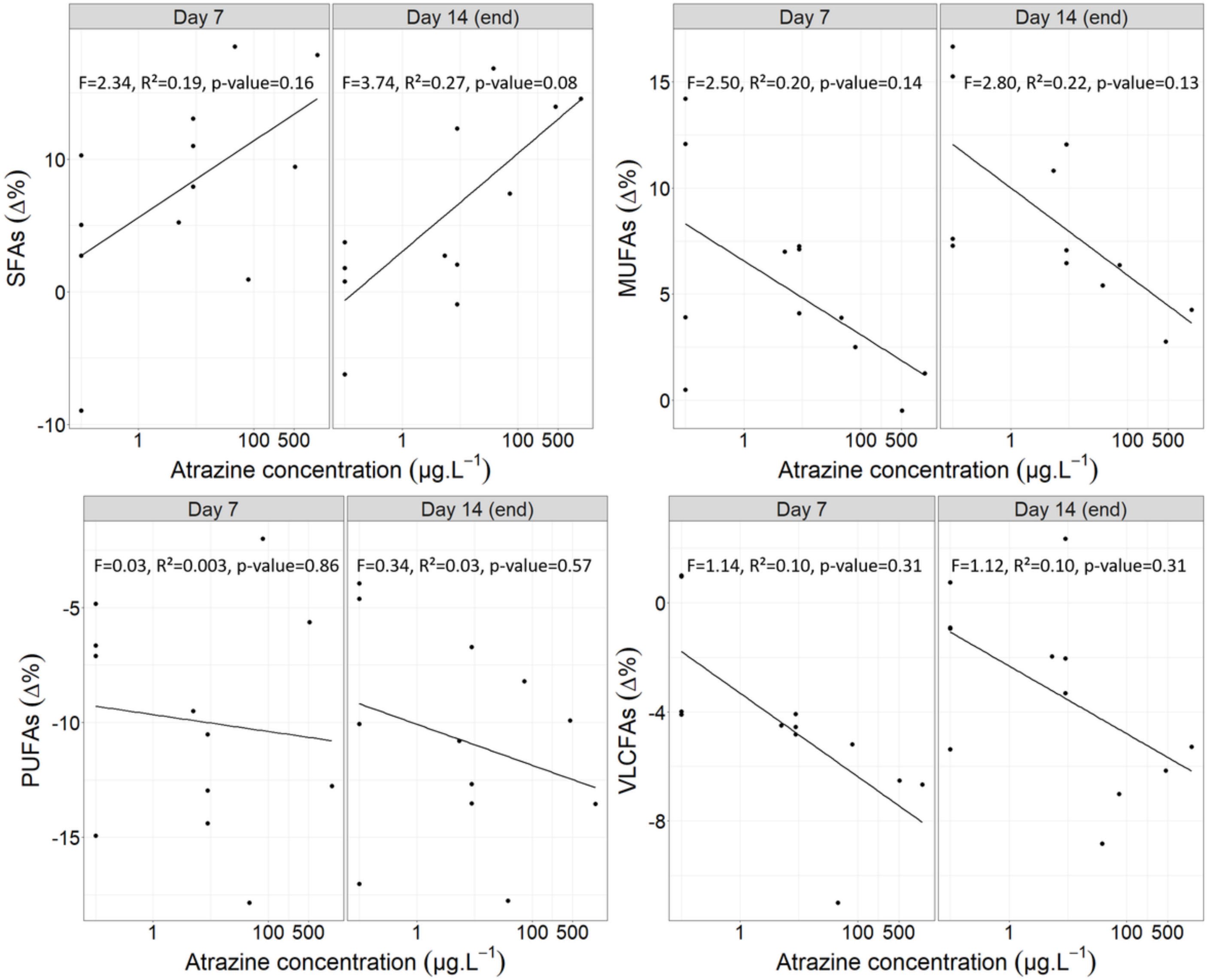

Figure 4

Linear regressions based on differences in proportions (Δ%) for the main fatty acid groups as a function of measured concentrations of atrazine (μg.L-1) after 7 days and 14 days of exposure

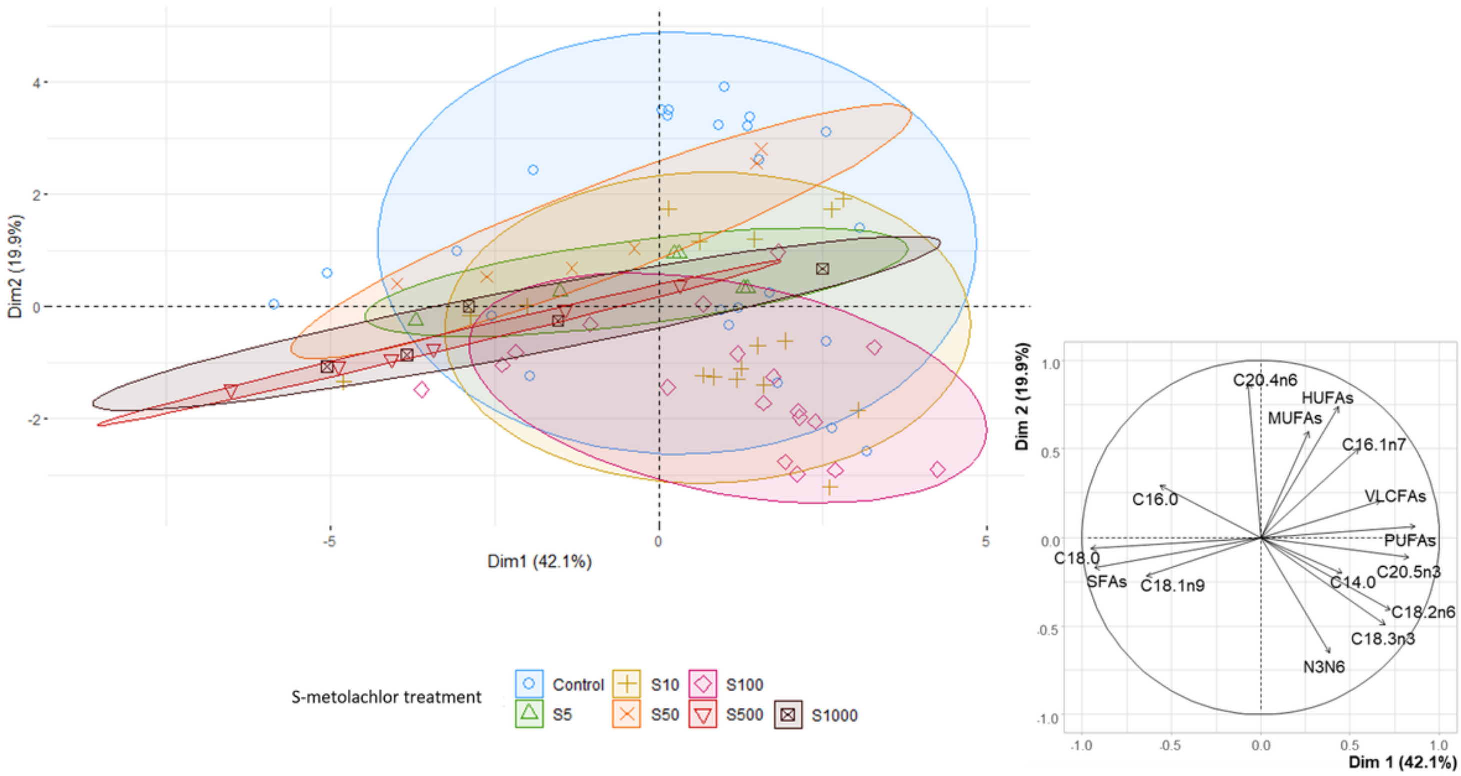

Figure 5

Principal component analysis (PCA) of fatty acid profiles at day 14 for the different S-metolachlor conditions. The left panel is the graph of individual FA and on the right panel corresponds to the circle of correlations. Ellipses have been plotted with a confidence level of 80%. All pseudo-replicates were considered to better represent the intra-condition variability

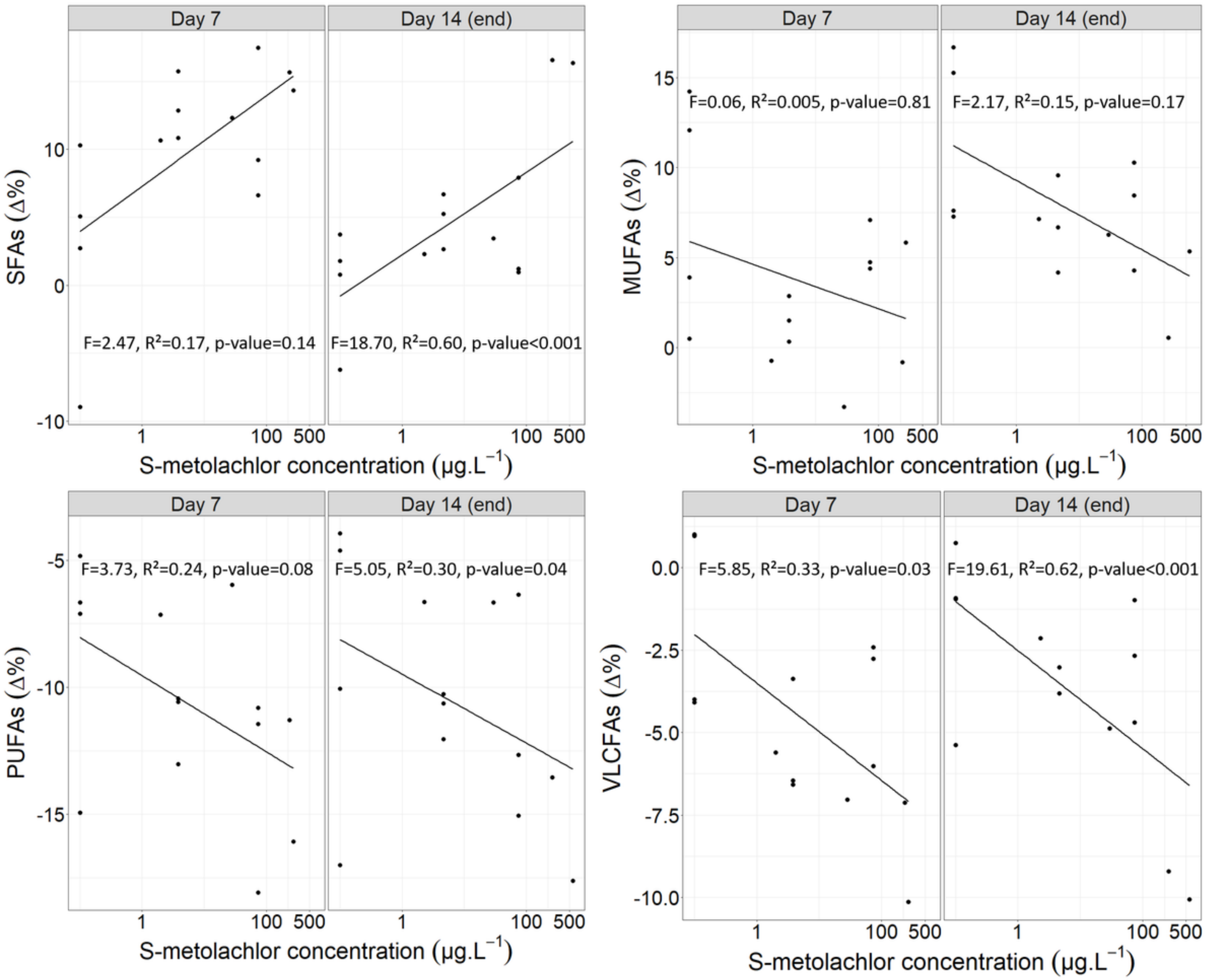

Figure 6

Linear regressions based on differences in proportions (Δ%) for the main fatty acid groups as a function of measured concentrations of S-metolachlor (µg.L-1) after 7 days and 14 days of exposure

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformationLauraMalbezinEffectsofatrazineandSmetolachloronstreamperiphytontaxonomicandfattyacidcomposition.docx